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BIOLOGICAL BULLETIN

THE FERTILIZATION REACTION IN ECHINARACHNIUS PARMA.

I. Cortical Response of the Egg to Insemination.

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Introduction.

So much has already been written about membrane "formation" in sea urchin eggs that one should perhaps hesitate to add to the list of papers on the subject. The observations here reported, however, would seem to fill a gap that has hitherto existed in a critical stage of the fertilization process. We know that the response of the egg to the spermatozoön on insemination first manifests itself in the appearance of a membrane at some distance from the vitellus—by some believed to be already on the uninseminated eggs; by others, to be actually formed *de novo*—but accounts as to why and how this membrane forms differ with different workers even where the same egg has been studied. Thus Herbst, Schücking, Kite and Heilbrunn among others have shown by different methods that the membrane is present on the unfertilized eggs; O. Hertwig originally at least expressed a similar view. R. Hertwig holds this view. On the other hand, Loeb, and the Loeb school generally not only believe that the membrane is formed after insemination but also that its formation is of great significance in the fertilization process; to Robertson, for example, membrane formation *is* fertilization. Again, we have the notion of Elder and of McClendon that the "fertilization membrane" is a precipitation membrane formed only in the presence of the intact jelly hull of the egg—a totally erroneous conception as shown by Harvey ('14). (See also, Lillie, '14.) Finally, while it is usually held that the membrane arises simultaneously from all points of the egg, suggestions have

not been wanting that the membrane forms as a wave that sweeps over the egg.

Fol first intimated that the membrane lifts from the egg as a wave dwelling on the rapidity with which the process is completed. Unfortunately he used eggs under pressure. Membrane formation as a progressive wave beginning at the site of sperm-entrance has been observed by Wilson who, however, is not at all sure. He says (in a footnote): "I have often observed that the formation of the membrane, in *Toxopneustes*, proceeds like a wave from the entrance-point around the periphery, but this is often irregular." Ries followed the fertilization with the cinematograph; he observed changes which indicated that the membrane forms first at one point.

In 1915 Dr. Tennent informed the writer that he had observed in cases a wavelike membrane formation. According to other workers, however, it would appear that no part of the cortex takes precedence in lifting off the membrane. Thus, Harvey ('10) who worked with *Toxopneustes*, *Hipponoë* and *Arbacia* says: "As observed in the living egg, almost immediately (1½ to 3 minutes) after addition of sperm the membrane substance becomes separated from the egg surface by spaces. These spaces fill with a fluid, unite and enlarge, thus pushing out the membrane some little distance.[1] This statement must refer to the egg of *Toxopneustes* (with which Wilson worked) or to that of *Hipponoë* for Heilbrunn after long experience with the egg of *Arbacia* was never able actually to follow the membrane elevation; he believed that in this egg the membrane lifted simultaneously from all parts of the cortex.

Loeb has followed membrane formation in the egg of *Strongylocentrotus purpuratus* by lowering the temperature of the sea-water which retards the process. He gives eight figures of the process described. "The beginning of the process shows itself in a roughening of the hitherto smooth surface of the egg. This is due to the formation of countless tiny vesicles which stand out on the surface of the egg. These small droplets quickly increase

[1] The reader must not conclude from this statement that Harvey believes that the unfertilized egg possesses a membrane. In this paper he makes a categorical statement that the membrane arises at fertilization. In his 1914 paper he reaffirms this statement.

in size (through absorption of water) and flow together into larger drops. This goes on until finally the contents of all the drops have run together into a continuous layer around the egg. Hence the surface lamellæ of the tiny droplets form later the fertilization membrane." At higher temperatures, the egg of *Strongylocentrotus* forms a membrane very quickly passing directly from the condition of the uninseminated egg to that with fully formed membrane and wide perivitelline space; thus too in *Arbacia*. (Loeb, *loc. cit.*)

During a study of the fertilization reaction in the egg of *Echinarachnius* covering several seasons at the Marine Biological Laboratory at Woods Hole, Mass., I have made observations on membrane elevation in this egg where the process though it takes place with great rapidity can nevertheless because of the size of the egg be followed with remarkable ease. I can, therefore, make the unqualified statement that in the egg of *Echinarachnius* membrane elevation proceeds as a wave from the entrance-point of the sperm around the cortex. Moreover, what is more significant, before membrane elevation cortical changes blocking farther sperm entry spread as a wave over the egg uniting finally at the point opposite the entrance-point of the sperm.

Observations.[1]

If eggs of *Echinarachnius* be inseminated with thin sperm suspension they throw off membranes that are fully formed and equidistant from the surface of the vitellus in from two to three minutes. This follows sperm penetration which may take place at any point. Sperm is engulfed by the egg in from fourteen to forty-five seconds after insemination; the membrane begins to lift off in from seven to twenty-two seconds after sperm entry is complete; and membrane elevation is complete in from nine to thirty seconds after it begins. The shortest time recorded from insemination to membrane elevation from all parts of the egg is thirty-nine seconds, all time being taken with a stop-watch of a

[1] All observations were made on eggs in a quantity of sea-water; the eggs never suffered compression. Though jelly-free eggs form membranes, most observations were made on eggs with jelly so that the eggs were therefore free from flattening when on the bottom of the dish—the jelly acting as a buffer. Flattening of the eggs might easily lead to erroneous conclusions.

standard make. In some thirty to sixty seconds after the membrane is off from the egg it fully rounds out, equidistant from the surface of the cytoplasm at all points. The following tables taken from the data give some idea of the time relations. The time is in seconds. The last column ("Membrane Off") gives the time at which the membrane is distinctly off the egg at all points but not yet fully rounded. Each record is from observations of a single egg.

TABLE I.

Insemination.	Sperm Engulfed.	Membrane Begins.	Membrane Off.
0	50	65	80
0	49	64	84
0	30	50	70
0	45	67	
0	30	50	70

TABLE II.

Insemination.	Sperm In.	Membrane Begins.	Membrane Off.
0	30	45	57
0	15	37	53
0		44	66
0		32	57
0		29	39
0		37	48
0		38	47

TABLE III.

Insemination.	Membrane Begins.	Membrane Off.
0	31	42
0	39	46
0	30	50
0	31	44
0	29	43
0	24	41
0	26	42
0	31	49
0	30	45
0	40	51
0	23	49
0	31	50
0	30	45
0	26	44
0	27	52
0	35	45
0	24	46

Average time of appearance of cone 90 seconds.

Average time of fully rounded membrane 134 seconds.

Immediately on insemination, sperm pierce the jelly hull, reaching the vitellus with rapid spiral movements; the moment the tip of the sperm touches the cortex of the vitellus all movements cease, the head and tail in a straight line at right angles to a tangent of the egg surface. Penetration follows as an activity of the egg; the spermatozoön does not bore its way in—the egg pulls it in.[2] After the head has disappeared within the cortex, membrane elevation begins.

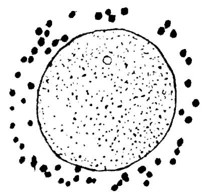
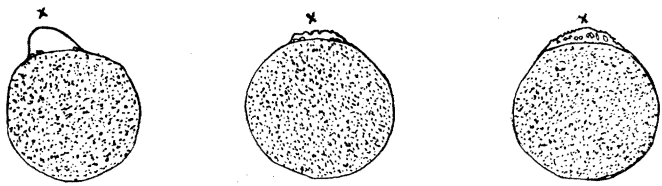


FIG. 1.[1] Freshly shed egg. Its pigmented jelly is as yet unswollen by the sea water.

After the sperm head has completely disappeared within the egg, the cortex reacts to penetration by pushing out a blister at the site of sperm entry (Fig. 2). This blister, which is not optically empty but contains minute drops that wander across this newly formed perivitelline space, may push off at once to a



2 3 4

FIG. 2. An egg ten seconds after the disappearance of the sperm head within the egg.

FIG. 3. Same as Fig. 2.

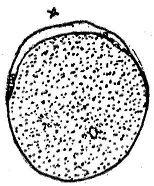
FIG. 4. Four seconds after the membrane began lifting. Free vesicles beneath the membrane.

distance equal to the greatest the membrane ever reaches (Fig. 2); or, what is more common, the membrane elevation slowly sweeps (Figs. 3, 4, 5, 6, 7, 8) around the surface of the egg increasing the width of the perivitelline space after having lifted off from the egg at all points (Fig. 9). One may picture the process thus: By escape of substances from the cortex at the

[1] Figs. 1, 9, 10, and 11 were drawn with the aid of a camera lucida. All others are of necessity free-hand sketches. Except in Fig. 1, the jelly hull is omitted. *x* marks the site of sperm entry.

[2] Kupffer and Benecke in 1878 made similar observations on the lamprey egg and reached the conclusion that the sperm is engulfed.

point of sperm entry the membrane thoroughly glued to the egg surface is pushed off; the material continuing throughout the cortex to escape progressively lifts off the membrane around the egg.[1] The droplets are squeezed out of the cortex into the perivitelline space and may reach the membrane as discrete bodies



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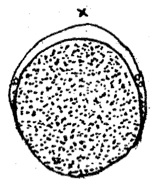

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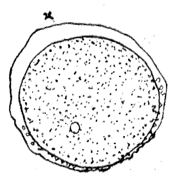
FIG. 5. About two seconds later than Fig. 4.
FIG. 6. Two to three seconds later than Fig. 5. Note vesicles forming.

before they fade from view. Often they go into solution more quickly. The membrane thickens slightly after formed.

As the membrane lifts off, it carries away any supernumerary sperm whose activity is in contrast to immobilized sperm previously engulfed by the egg. Not only is it true that at no



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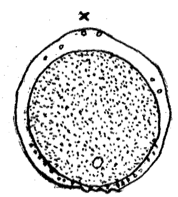

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FIG. 7. About two seconds later than Fig. 6. Membrane incomplete at pole opposite entrance-point of the sperm.

FIG. 8. Twelve seconds after beginning of membrane lifting. The membrane is fully formed but still wrinkled, especially at point at which it lifted last.

point at which the membrane has elevated can sperm enter, but also that membrane elevation at a given point though not complete for the whole egg is a bar to sperm entrance at any point on the egg surface. And this is the most significant point in the reaction. *Before the actual elevation of the membrane, some cortical change beginning at the point of sperm entry sweeps over*

[1] This does not mean, however, that the eggs decrease in size after fertilization.

the egg immunizing it to other sperm; the direct opposite pole of the site of sperm entry is the last point affected.

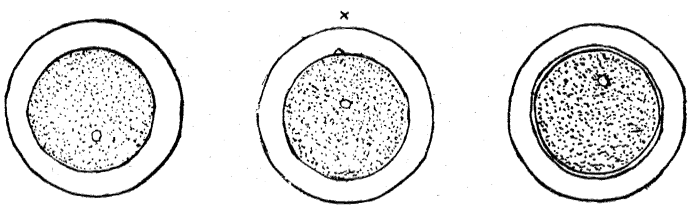
The site of sperm entry becomes a "point of injury" and is "negative" for any other sperm arriving at this point, all other portions around the egg being positive. This "wave of negativity" moves over the egg, its rate varying with the variations in the time that marks the disappearance of the sperm within the cytoplasm. When only the tip of the sperm head has entered the cytoplasm, the immediate vicinity of the site of penetration alone can not engulf sperm. As more of the sperm head disappears within the cortex of the egg, the "negativity" of the cortex for sperm entry progresses still farther around the egg until at the moment the head has disappeared the egg can engulf sperm only at one point—the pole opposite that at which the sperm entered the egg. A "wave of negativity" thus progressively sweeps over the egg from the point of sperm entry *preceding the actual beginning of membrane lifting.* Before the membrane begins lifting at the site of sperm entry sperm can no longer enter at any point on the egg.[1] From the point of sperm entry a definite gradient of membrane elevation is established, the last point of membrane elevation being at the pole opposite that of successful sperm entry. This gradient, therefore, follows that of diminished susceptibility to sperm penetration initiated at the entrance-place of the sperm.

If several spermatozoa become attached to the vitellus at the same instant membrane elevation starts at each point of attachment no matter what the distance separating them. Thus, two, three or more sperm may be attached at very nearly the same point or at varying distances from each other; in these cases other sperm later in arrival at the periphery of the egg, do not become engulfed, though they may be affixed by the egg. The membrane lifts at these points as blisters; each point behaves with reference to the space between it and an adjacent point of attachment as does the point in monospermic eggs, except that the "waves of negativity" spreading from each move to meet

[1] In 1878 O. Hertwig expressed the view that it is the egg plasma itself, if its vitality be unimpaired, which alone can prevent the entrance of more than one sperm.

thus diminishing the positive reaction of the cortex between two sperm points. Often, of course, even in heavy insemination a single spermatozoön reaches the egg at some one point ahead of others; in this case the egg is monospermic and so behaves. The observer is indeed struck with the rare occurrence of polyspermy even with heavy insemination.

When a spermatozoön becomes attached not only the penetration of other sperm but also their fixation to the vitellus depends upon the degree of penetration of the attached sperm or perhaps rather on the rate at which the "wave of negativity" is propagated around the egg. Thus, at the beginning of penetration, sperm nearby cannot become attached to the vitellus;



9 10 11

FIG. 9. Two minutes after insemination. Membrane is fully rounded, equidistant at all points from the now spherical egg.

FIG. 10. About same time as in Fig. 9. Cone is clearly visible.

FIG. 11. Ten minutes after insemination. Egg with hyaline plasma layer formed

those farther removed may become attached, lashing back and forth very vigorously. The heads of those still farther away but not far enough beyond the area of decrement actually to be engulfed are likewise affixed and present a swollen appearance. When the membrane lifts off these affixed sperm are carried with it.

The *Echinarachnius* egg, then, would seem to be unusual, since a gradient of susceptibility to the sperm may actually be demonstrated in the cortical changes following sperm entry.

After elevation of the membrane in *Echinarachnius* egg a cone (Fig. 10) of clear cytoplasm forms at the point of sperm entry; sectioned eggs show the sperm within where rotation of the head may begin. This cone[1] disappears; within some ten to fifteen

[1] The cone and the behavior of sperm that are attached to the cortex but do not enter constitute important checks on the observation of the point of sperm entry.

minutes after insemination a thin film forms on the surface of the cytoplasm (Fig. 11) the hyaline plasma layer, or gelatinous film of Loeb.

Experiments are being made at present with the hope of analyzing farther these cortical responses of the *Echinarachnius* egg to the sperm. We may, however, point out in conclusion to these observations that in the progressive membrane lifting and the cortical change that precedes it we have another of the numerous examples of an activity gradient; this of course is distinct from the primary axial (polar) gradient that exists in echinoderm eggs (see Child). In following these waves of cortical change we are actually viewing the propagation of the effect of a stimulus in an irritable cell. One effect of this stimulus of sperm entrance is to alter the plasma to such an extent that it prevents the entrance of other sperm. It is not the membrane that is a block to polyspermy; that block exists before the membrane lifts off. The membrane is merely the sign and consequence of more profound cortical changes.[1] In a way, it is of no significance that the membrane rises sharply into visibility provided these primary cortical changes take place. And indeed I have seen any number of eggs fertilize and develop without throwing off membranes. Such eggs are as incapable of reinsemination as eggs that have formed membranes. In the *Echinarachnius* egg, then, normal development has been already initiated by the sperm when the membrane begins to form.

[1] Says Lillie: "The fundamental mechanism for the prevention of polyspermy is the neutralization of the fertilizin by the anti-fertilizing present in the egg; *i. e.*, the occupancy of the spermophile side-chain of the fertilizin by the anti-fertilizin.

"The question may be raised why such neutralization of the fertilizin is delayed until the moment of fertilization? The answer to this difficulty is fairly clear. The fertilizin is located in the cortex of the egg, and the anti-fertilizin is more deeply situated; they therefore do not interact so long as the cell body as a whole is quiescent. But as soon as the cortical fertilizin becomes activated by union with the sperm it at once begins to attach certain substances in the egg, as demonstrated in the third part of this paper; this sets up diffusion evidenced by escape of pigment, and by cytoplasmic flowing, and the two substances are brought together and interact. While this explanation is partly hypothetical, the spatial separation of the fertilizin and anti-fertilizin and the quiescent character of the cell-body in the unfertilized egg are facts; so also are the movements of diffusion and the cytoplasmic currents set up on fertilization."

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